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Annals of Biological Research, 2011, 2 (5) :191-195
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Callus production under different culture medium in *Pluchea lanceolata*: A perennial medicinal plant

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ABSTRACT

This research evaluated effect of different plant culture media on callus induction on Pluchea lanceolata. P. lanceolata is an important medicinally plant species that is rich in secondary metabolites like quercetin, β-sitosterol, triterpenol, etc. Four different types of culture media were employed to determine callus responses viz. MS_1 (Murashige and Skoog medium), MS_2 (Woody Plant medium), MS_3 (Wood & Braun medium) and MS_4 (White's medium). Each medium was supplemented with 6-Benzyladanine (BA; 2 mg L^{-1}) and α-Naphthalene acetic acid (NAA; 2 mg L^{-1}) and tested for their influences. There are differences in callus initiation and morphology among the media. MS_1 medium showed better responses in terms of fresh weight, dry weight, water content and chlorophyll content as compared to other medium used.

Key words: *Pluchea lanceolata*, Murashige and Skoog medium, Woody Plant Medium, Wood & Braun Medium, White's medium.

INTRODUCTION

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. Many valuable medicinal plants are under the verge of extinction. The red data book of India has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species [1].

Pluchea lanceolata, commonly known as Rasana, is a perennial medicinal herb grown in warm climatic regions of India. *Pluchea* is a genus of flowering plant in the *Asteraceae* family. The plant contains high amounts of medicinally important secondary metabolites (quercetin, β-sitosterol, triterpenol, *etc*.,) which give it anti inflammatory and analgesic properties [2-5]. Of these, quercetin (a flavonoid) is considered as active ingredient which has many biological roles including anti-inflammatory, anti-cancerous, anti-bacterial, anti-viral, antigonadotropic and antihepatotoxic activities. It is widely used in the treatment of rheumatoid arthritis in the indigenous system of medicine [6]. Due to unviable seed production and unscrupulous collection, the wild population of plant species has become vulnerable to extinction. However, the plant species has been listed as a priority species by Ministry of Health and Family Welfare, Government of India, therefore measures are needed to conserve it using the tissue culture techniques. The research objectives were to study suitable concentration of plant growth regulators and medium for Rasana callus production and to investigate responses on callus production. By achieving these objectives we believed we would have advanced our knowledge base that could facilitate photochemical production and extraction of its active principle.

MATERIALS AND METHODS

P. lanceolata was collected from the wild regions of Luni, Jodhpur. Leaf explants were excised from the young plants, washed in running tap water for 30 min and then in liquid detergent solution (labolene) for 5 min. They were then surface sterilized with 0.1% mercuric chloride solution for 2 min and thoroughly washed 4-5 times with sterile distilled water. The explant was dissected into small pieces and cultured on different culture media. Four different types of culture media were employed (Table 1) to determine callus responses viz. MS_1 [7], MS_2 (Woody Plant Medium) [8], MS_3 [9] and MS_4 (White's medium) [10].

Table 1 Basal nutrient medium composition (mg L^{-1}) of Murashige and Skoog medium (MS_1), woody plant medium (MS_2), Wood and Braun medium (MS_3) and White's medium (MS_4)

	Media			
Constituents (mg L^{-1})	MS_1 (mg L^{-1})	MS_2 (mg L^{-1})	MS_3 (mg L^{-1})	MS_4 (mg L^{-1})
Sucrose	30,000.00	30,000.00	20,000.00	20,000.00
Glycine	400.00	2.00	3.00	3.00
Myo-inositol	20,000.00	100.00	100.00	--
Cysteine	--	--	--	0.10
Thiamine. HCl	100.00	1.00	0.10	0.10
Pyridoxine. HCl	100.00	0.50	0.10	0.10
Nicotinic acid	100.00	0.50	0.50	0.50
Na_2.EDTA.$2H_2O$	7460.00	37.30	--	--
Pentothenic acid	--	--	--	1.00
2, 4-D	--	--	--	6.00
KCl	--	--	65.00	65.00
$MgSO_4$. $7H_2O$	7400.00	180.69	360.00	720.00
Na_2HPO_4. H_2O	--	--	16.50	16.50
$CaCl_2$. $2H_2O$	8800.00	96.00	--	--
KNO_3	38,000.00	--	80.00	80.00
NH_4NO_3	33,000.00	400.00	--	--
KH_2PO_4	3400.00	170.00	--	--
Ca $(NO_3)_2$. $4H_2O$	--	386.34	200.00	300.00
$FeSO_4$. $7H_2O$	5560.00	--	--	--

$MnSO_4$. $4H_2O$	4460.00	22.30	--	7.00
$MnCl_2$. $4H_2O$	--	--	4.50	--
Na_2SO_4	--	--	88.00	200.00
KI	--	--	0.75	0.75
K_2SO_4	--	990.00	--	--
$CoCl_2$. $6H_2O$	5.00	--	--	--
$ZnSO_4$. $7H_2O$	1720.00	8.60	1.50	3.00
$CuSO_4$. $5H_2O$	5.00	0.25	0.013	--
H_3BO_3	1240.00	6.20	1.50	1.50
$FeCl_3$. $6H_2O$	--	--	2.50	--
Na_2MoO_4. $2H_2O$	50.00	0.25	--	--
Fe $(SO_4)_3$. $7H_2O$	--	27.80	--	2.50

The basic salts of all the culture media were supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar-agar powder with plant growth regulators (PGRs). The concentrations of PGRs used for callus induction and establishment were: 6-Benzyladanine (BA; 2 mg L^{-1}) and α-Naphthalene acetic acid (NAA; 2 mg L^{-1}). The pH of the media was adjusted 5.8 to 5.85 and autoclaved at 15 psi for 15 min. Cultures were maintained at 25±2ºC under 16 h photoperiod illuminated by fluorescent light (2000-3000 lux) and 55±5% relative humidity. Established unorganized callus cultures grown on each medium with NAA (2 mg L^{-1}) and BA (2 mg L^{-1}) of 30 days were taken up for determination of fresh weight, dry weight, water content and chlorophyll content [11].

RESULTS AND DISCUSSION

This research evaluated influence of different culture media on callus initiation. Scanty information was reported for the medium optimization in this plant species for callus induction. Kumar *et* al. [12] used WB medium and Arya *et al.* [13] used MS medium for callus induction. Therefore, four different plant tissue culture media along with NAA (2 mg L^{-1}) and BA (2 mg L^{-1}) were tried for callus induction from leaf explants. Among the various media tried MS_1 was found to be best for producing fluorescent green, fast growing and friable callus (Figure 1). MS_2 and MS_3 gave brown, slow growing and hard callus whereas MS_4 showed poor callus growth, though the colour of callus was green.

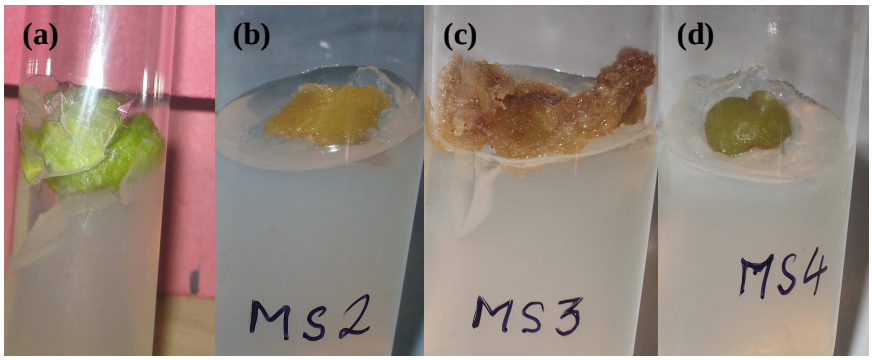

Figure 1 *Pluchea lanceolata* callus response on Murashige and Skoog medium (MS_1), Woody plant medium (MS_2), Wood and Braun medium (MS_3) and White's medium (MS_4) after 30 days of inoculation.

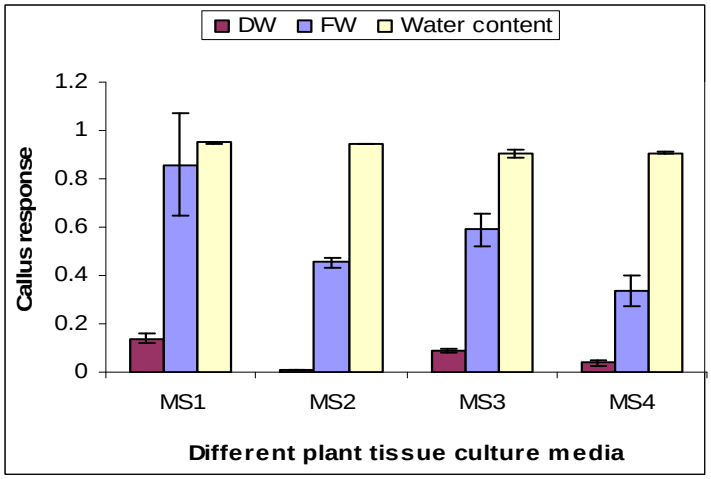

Figure 2 *Pluchea lanceolata* callus responses (fresh weight, dry weight ans water content) on Murashige and Skoog medium (MS_1), Woody plant medium (MS_2), Wood and Braun medium (MS_3) and White's medium (MS_4) after 30 days of inoculation. Each value represents the mean ± SE of three replicates.

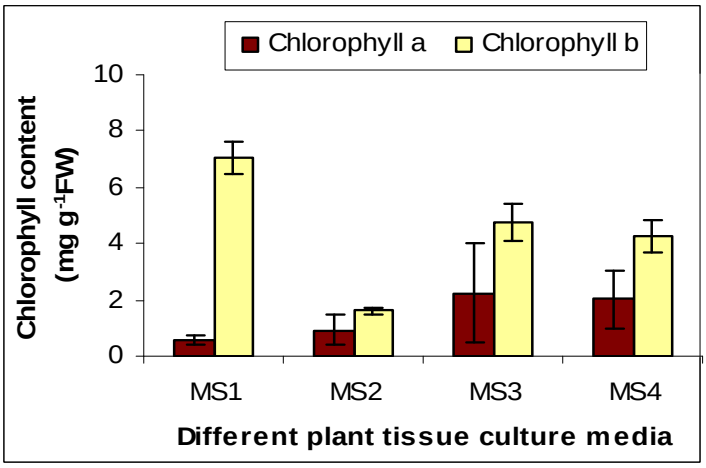

Figure 3 Chlorophyll content of *Pluchea lanceolata* grown on Murashige and Skoog medium (MS_1), Woody plant medium (MS_2), Wood and Braun medium (MS_3) and White's medium (MS_4) after 30 days of inoculation. Each value represents the mean ± SE of three replicates.

Figure 2 depicts the fresh weight (g per explant), dry weight (g per explant) and calli water content. Among the various culture media employed, MS_1 recorded significantly higher FW, DW and water content as compared to others. Increase in fresh weight might be due to simultaneous accumulation of dry mass and water uptake which further enhanced DW. During the growth period reserve material accumulation occurs with such intensity that fresh weight goes on increasing in spite of the decrease in water content as reported by Nedeva and Nikolova [14] in wheat. According to Kermode and Bewley [15], a rapid increase in fresh weight and water content occurs during histo-differentiation and early cell expansion as reported in the present study.

Figure 3 describes the content of photosynthetic pigment *i.e.* chlorophyll a and chlorophyll b. Significant results were seen in case of MS_1, both chlorophyll a and chlorophyll b content was higher than the other culture media tested. The results of the photosynthetic pigment coincide with that of FW and water content of the calli. Ali *et al.* [2] conducted phytochemical studies on the aerial parts of *P. lanceolata* and found rise in chlorophyll content. Higher chlorophyll accumulation was reported in *Chlorophytum borivilianum* and *Terminalia bellerica* due to better growth performance in the culture media [16]. Therefore, present findings will help in defining easier approaches for high rate multiplication in *P. lanceolata*. Callus cultures can further be exploited for the large scale production of active principle present in this important medicinal plant.

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